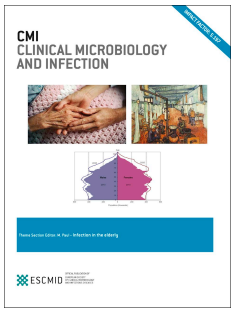
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Journal Pre-proof



Post-COVID-19 syndrome and humoral response association after one year in vaccinated and unvaccinated patients

Maddalena Peghin, Maria De Martino, Alvisa Palese, Valentina Gerussi, Giulia Bontempo, Elena Graziano, Erica Visintini, Denise D' Elia, Fabiana Dellai, Francesco Marrella, Martina Fabris, Francesco Curcio, Assunta Sartor, Miriam Isola, Carlo Tascini

PII: S1198-743X(22)00155-0

DOI: https://doi.org/10.1016/j.cmi.2022.03.016

Reference: CMI 2888

To appear in: *Clinical Microbiology and Infection*

Received Date: 31 December 2021

Revised Date: 9 March 2022

Accepted Date: 12 March 2022

Please cite this article as: Peghin M, De Martino M, Palese A, Gerussi V, Bontempo G, Graziano E, Visintini E, Elia DD', Dellai F, Marrella F, Fabris M, Curcio F, Sartor A, Isola M, Tascini C, Post-COVID-19 syndrome and humoral response association after one year in vaccinated and unvaccinated patients, *Clinical Microbiology and Infection*, https://doi.org/10.1016/j.cmi.2022.03.016.

Which is the role of vaccination and umoral response in post-COVID syndrome?

Methods

Results >>>>>>>>>>

SARS-CoV2 infection

6 months

12 months

* RBD, receptor binding domain

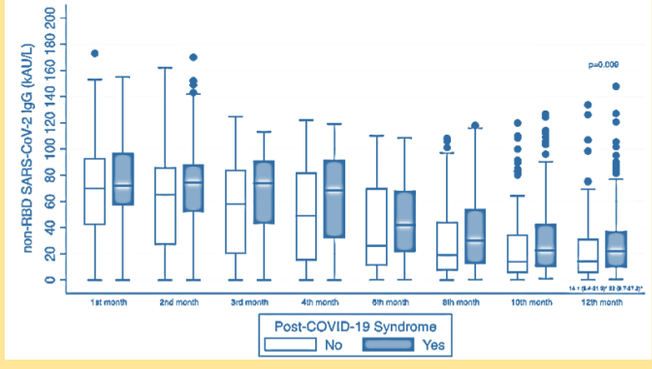
Phone interview → **Symptoms?**

Serological samples
- → **Non-RBD* SARS-CoV-2 IgG** — *Response to natural infection*
- → **RBD* SARS-CoV-2 IgG** — *Response to vaccination and natural infection*

Vaccination status against SARS-CoV-2
- Vaccinated
- Unvaccinated

479 individuals (52.6% female); overall prevalence of post-COVID-19 syndrome at 12 months: 47.2%

Symptoms	*p=0.209*	
Worsened	22.7 %	15.8 %
Unchanged or Unaffected	65.9 %	71.2 %
Improved	11.4 %	13.0 %



Association between non-RBD IgG and post-COVID syndrome (p=0.003)
No link between RBD IgG and post-COVID syndrome (p=0.441)

The SARS-CoV-2 vaccination is not associated with worsening or the appearance of post-COVID-19 symptoms one year after the acute infection.

Persistence of high titers serological response induced by natural infection but not by vaccination may play a role in long-COVID-19.

Post-COVID-19 syndrome one year after acute infection in vaccinated and unvaccinated patients and the association with humoral response.
Peghin M., De Martino M., Palese A., Gerussi V., Bontempo G., Graziano E., Visentini E., D' Elia D., Dellai F., Marrella F., Fabris M., Curcio F., Sartor A., Isola M., Tascini C.

Post-COVID-19 syndrome and humoral response association after one year in vaccinated and unvaccinated patients

Maddalena Peghin [1,2], Maria De Martino [3], Alvisa Palese [4], Valentina Gerussi [1], Giulia Bontempo [1], Elena Graziano [1,2] Erica Visintini [4], Denise D' Elia [1], Fabiana Dellai [1], Francesco Marrella [1], Martina Fabris [5], Francesco Curcio [5], Assunta Sartor [5], Miriam Isola [3], Carlo Tascini [1]

Authors

[1] Infectious Diseases Division, Department of Medicine, University of Udine and Azienda Sanitaria Universitaria Friuli Centrale, 33100, Udine, Italy

[2] Infectious and Tropical Diseases Unit, Department of Medicine and Surgery, University of Insubria-ASST-Sette Laghi, Varese, Italy.

[3] Division of Medical Statistic, Department of Medicine, University of Udine, 33100 Udine, Italy

[4] School of Nursing, Department of Medical Sciences, University of Udine, 33100 Udine, Italy

[5] Institute of Clinical Pathology, Department of Laboratory Medicine, *Azienda Sanitaria Universitaria Friuli Centrale* and University of Udine, Department of Medicine, 33100, Udine, Italy

Corresponding author:

Maddalena Peghin MD PhD

Clinica di Malattie Infettive, *Azienda Sanitaria Universitaria Friuli Centrale,* Presidio Ospedaliero Universitario Santa Maria della Misericordia

Piazzale Santa Maria della Misericordia 15 33010 Udine, Italy.

Telephone number: + 39 0432555350

E-mail address: maddalena.peghin@gmail.com

Running title: Post COVID syndrome, vaccination and humoral immunity

Word count (manuscript):

Keywords: post-COVID-19; COVID-19 survivors; long COVID-19; SARS-CoV-2 antibodies; SARS-CoV-2 serology; natural immunity; hybrid immunity; SARS-CoV-2 vaccination; COVID-19 vaccination; vaccinated; unvaccinated

Abstract

Objectives. To describe the impact of vaccination and the role of humoral responses on post-coronavirus disease 2019 (COVID-19) syndrome one year after the onset of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Methods: A prospective study. Interviews investigated post-COVID-19 syndrome 6 and 12 months after the disease onset of all adult in- and outpatients with COVID-19 attending Udine Hospital (March–May 2020). Vaccination status and two different serological assays to distinguish between response to vaccination (receptor-binding domain –RBD SARS-CoV-2 IgG) and/or natural infection (non-RBD- SARS-CoV-2 IgG) were also assessed.

Results. 479 individuals (52.6% female, mean age 53 years) were interviewed 13.5 months (0.6 SD) after acute infection. Post-COVID-19 syndrome was observed in 47.2% (226/479) of patients after one year. There were no significant differences in the worsening of post-COVID 19 symptoms (22.7% vs 15.8%, p = 0.209) among vaccinated (n=132) and unvaccinated (n=347) patients. The presence of non-RBD SARS-CoV-2 IgG induced by natural infection showed a significant association with post-COVID-19 syndrome (OR 1.35, 95% CI 1.11–1.64, p = 0.003), and median non-RBD SARS-CoV-2 IgG titres were significantly higher in long-haulers than in patients without symptoms 22 (IQR 9.7–37.2) vs 14.1 (IQR 5.4–31.3) kAU/L, p = 0.009) after one year. In contrast, the presence of RBD SARS-CoV-2 IgG was not associated with the occurrence of post-COVID-19 syndrome (> 2500 U/mL vs 0.9–2500 U/mL, OR 1.36, 95% CI 0.62–3.00, p = 0.441) and RBD SARS-CoV-2 IgG titres were similar in long-haulers than in patients without symptoms (50% values > 2500 U/mL vs 55.6% values > 2500 U/mL, p = 0.451)

Conclusions. The SARS-CoV-2 vaccination is not associated with the emergence of post-COVID-19 symptoms over one year after acute infection. The persistence of high serological titres response induced by natural infection but not by vaccination, may play a role in long-COVID-19.

INTRODUCTION

Post-coronavirus disease 2019 (COVID-19) syndrome is a heterogeneous, multisystemic, post-acute sequelae impacting health and the quality of life of all ages (1-3). The potential pathophysiological mechanisms are unknown and may encompass a complex interaction between the virus-specific cytopathic effects, the inflammatory damage, the allo- and autoimmune responses to the acute infection on the one hand, and the expected sequelae of post-critical illness due to organ and microvascular damage on the other (4). To date, there is still a gap on how natural immunity and hybrid immunity, which refer to the immune-strengthening effect of exposure to infection followed by vaccination, function in post-COVID 19 (5-7). A few studies available have suggested both a potential improvement and deterioration of post-COVID-19 symptoms after vaccination in previously infected patients and variable associations between humoral responses and post-COVID-19 syndrome after the natural infection (8-11). Investigating immunological mechanisms could inform both clinical and public health decisions regarding the prevention of and the potential tailored treatments for long COVID-19 (4). Thus, the aim of this study was to describe the post-COVID-19 syndrome one year after the acute infection by focusing (a) on the influence of vaccination on long-term symptoms, and (b) on the role of humoral responses among survivors with natural and hybrid immunity.

METHODS

Study design and patients

A prospective study (5) reported according to the Strengthening the Reporting of Observational Studies in Epidemiology Statement (Supplementary Table 1). Those eligible were: (a) all adults (≥ 18 years) diagnosed with COVID-19 during the first wave (March–May 2020) and cared for by an Academic Hospital in all settings (b) followed up at 6 (September–November 2020) and at 12 months (March–May 2021); and (c) willing to participate (Figure 1).

Data collection

Demographic and clinical databases were populated at the enrolment and over time (Supplementary Table 2). Participants were telephone-interviewed by the same trained nurses at 6 and 12 months using a homogeneous questionnaire, pilot-tested and previously validated (5), investigating persistent or emerging symptoms potentially associated with COVID-19, as expressed by patients' own words (12). Post-COVID-19 syndrome was defined as signs and symptoms developed during or following an infection consistent with COVID-19, continued for more than 12 weeks, and not explained by an alternative diagnosis (13). Signs/symptoms reported by patients were classified by four

independent researchers (Supplementary Table 3) and then matched between the first and the second interview in order to check changes, if any, over time. Therefore, patients were classified as: (a) unaffected when asymptomatic at both follow-ups; (b) unchanged when symptoms remained the same; (c) worsened when new symptoms emerged; and (d) improved, when symptoms were recovered/resolved (5).

In Italy, the SARS-CoV-2 vaccination campaign started on 27 December 2020. Vaccines approved were those with Adenovirus Vector (ChAdOx1 nCoV-19 Oxford–AstraZeneca and Ad26.COV2.S Janssen COVID-19 vaccine) and the mRNA (BNT162b2 Pfizer–BioNTech and mRNA-1273 Moderna). Thus, at 12 months, patients were asked to communicate vaccination state (yes/no) by also reporting the date and type of vaccine received. Data collected were matched in their accuracy with electronic health records; then, patients were categorized as vaccinated if they had received the vaccine at least 2 weeks before the interview; those with combined immunity from natural SARS-CoV-2 infection and vaccination were considered to have hybrid immunity. Bias has been prevented, as reported in Supplementary Table 4.

Antibody measurement and other laboratory methods

SARS-CoV-2 antibody measurement were performed in a subgroup of patients (n=546) who agreed to participate in a parallel study (CORMOR 3-4®) (14): their serological data at the time of the interview (+/-2 months) were recorded in the database (Figure 1). The role of serological response in post-COVID-19 syndrome was assessed by using two antibody assays with different abilities to recognize the receptor-binding domain (RBD) of the Spike protein as the main target stimulated by the SARS-CoV-2 vaccination. Specifically, an IgG test that is not able to recognize the RBD SARS-CoV-2 protein (iFlash-SARS-CoV-2; IgG positivity cut-off > 10.0 kAU/L) was used to follow natural humoral response (non-RBD IgG) and an IgG test of the SARS-CoV-2 S protein RBD (Elecsys Roche; IgG positivity cut-off (< 0.9 U/mL and maximum value > 2500 U/mL) was used to follow both natural and vaccine-induced humoral response to compare vaccinated and unvaccinated patients (Figure 1). Laboratory methods used are detailed in Supplementary Table 5.

Ethical issues

The reference Ethics Committee of Friuli Venezia Giulia (CEUR-2020-OS-219 and CEUR-2020-OS-205) approved the study.

Statistical analysis

Patients were divided into two groups (vaccinated, unvaccinated) at the time of the interview at 12 months. The Shapiro–Wilk test was used to assess whether data were normally or non-normally distributed. Categorical variables were compared using the chi-square (χ2) test or Fisher's exact test, while quantitative variables were compared using the t-test or Mann–Whitney U test, as appropriate. A univariable and multivariable logistic regression was performed to explore features associated with post-COVID-19 syndrome, estimating the odds ratio (OR) at 95% Confidence Interval (CI), (STATA 17.0).

RESULTS

Acute COVID-19 onset and post-COVID-19 syndrome after one year

Overall, during the first wave, 1,067 COVID-19 patients were diagnosed in our hospital. Of them, 599 attended the 6-month interview and 479 the 12-month interview (Figure 1). Their baseline characteristics and clinical data at the COVID-19 onset are reported in Table 1 and 2. At a median of 13.5 months (0.6 SD) after the acute COVID-19 onset, the prevalence of the post-COVID-19 syndrome was 47.2% (226/479) (95% CI 42.64–51.76), which was higher than at 6 months (40.2%, 241/599) (95% CI 36.38–44.28) (Table 2).

Overall, among patients reporting post-COVID-19 symptoms at 6 months (201/479; 42.0%), 29.8% of them reported improvements at 12 months, while 70.2% declared unchanged symptoms. Of note, 85 (30.6%) patients reported the onset of new post-COVID-19 symptoms at 12 months.

Specifically, there was a significant increase in rheumatological (6.3% vs 12.7%, $p = 0.002$), ocular (0.3% vs 23%, $p < 0.001$) and psychiatric symptoms (4.8% vs 10.2%, $p = 0.006$), whereas there was a significant decrease in neurological (9.5% vs 2.7%, $p < 0.001$) and cutaneous symptoms (3.5% vs 1.2%, $p = 0.047$) at 12 months compared with 6 months (Figure 2).

Post-COVID-19 syndrome in vaccinated and unvaccinated patients

Overall at the time of the interview, 347 (72.4%) patients were unvaccinated, 132 were vaccinated (132, 27.6%) with at least one dose and 111 had already received the second dose (all mRNA type). Patients received the first and second vaccine dose at a mean of 12.4 (1.9 SD) and 13.5 (2.3 SD) months respectively after the onset of acute COVID-19. The time between the vaccination (first or second dose) and the interview ranged from 15 to 140 days.

As reported in Tables 1 and 2, vaccinated patients were more frequently female (94/132, 71.2%) and health-care workers (HCWs) (73/120, 60.8%), with a less severe disease at acute onset (105/132, 79.5% mild or asymptomatic). In both groups, there were patients still suffering from post-COVID-19-symptoms at 6 months, but those unvaccinated had reported higher rates of symptoms at 6 months compared to those who were vaccinated (45.2% vs 33.3%, p = 0.018). As reported in Table 3, post-COVID-19 symptoms varied between 6 and 12 , according to the vaccination status: in both groups, there were patients whose symptoms had worsened (22.7% vs 15.8%) or improved (11.4% vs 13.0%), although most commonly, patients reported unchanged symptoms or to be unaffected (65.9% vs 71.2%).Overall, these differences were not statistically significant except for the improvement in hair loss among unvaccinated (p = 0.033) and the worsening ocular symptoms among those vaccinated patients (p = 0.021). No significant difference in post-COVID-19 syndrome at 12 months emerged according to the vaccine received (45.8% mRNA vaccine and 12.5% Adenovirus Vector vaccine, p = 0.137) and the vaccination status (38.1% incomplete and 45.9% complete, p=0.507). Multivariable analyses of post-COVID-19 syndrome risk factors associated are reported in Supplementary tables 6 and 7.

Post-COVID-19 syndrome and antibody response after natural infection and vaccination

Patients included in the CORMOR 3-4 study were monitored (Figure 1) and the antibody response of non-RBD SARS-CoV-2 IgG over time from symptom onset is shown in Figure 3. Overall, 275 patients completed the serological follow-up with non-RBD SARS-CoV-2 IgG in proximity of the 12-month interview after the onset of acute COVID-19 and 102 patients underwent a serological test with RBD SARS-CoV-2 IgG (Figure 1).

About 153 of the 275 patients (55.6%) maintained non-RBD SARS-CoV-2 IgG after one year. The median value of non-RBD SARS-CoV-2 IgG titre was about 22 kAU/L (IQR 9.7–37.2 kAU/L). The presence of non-RBD IgG induced by natural infection was significantly associated with the occurrence of post-COVID-19 syndrome (OR 1.35, 95% CI 1.11–1.64, p = 0.003), and the median non-RBD SARS-CoV-2 IgG was significantly higher in long-haulers than in patients without symptoms 22 (IQR 9.7–37.2) vs 14.1 (IQR 5.4–31.3) kAU/L, p = 0.009) (Figure 3 and Table 4).

In contrast, the presence of RBD SARS-CoV-2 IgG in patients with hybrid immunity compared with those with natural immunity was not linked with the development of post-COVID-19 syndrome (> 2500 U/mL vs 0.9–2500 U/mL, OR 1.36, 95% CI 0.62–3.00, p = 0.441) and RBD SARS-CoV-2 IgG titres were similar in long-haulers and in patients without symptoms (50% values > 2500 U/mL vs 55.6% values > 2500 U/mL, p = 0.451). The antibody response among vaccinated and unvaccinated patients is shown in Table 4.

DISCUSSION

The results of this prospective study indicate that: (a) post-COVID-19 syndrome rates are high up to one year after acute infection; (b) receiving the SARS-CoV-2 vaccination is not associated with worsening post-COVID-19 symptoms; and (c) the persistence of a high titre serological response induced by natural infection but not by vaccination may play a role in long COVID-19. Our findings support the practice of offering SARS-CoV-2 vaccination regardless of infection history and identifies a novel aspect of humoral response in patients with post-COVID-19 syndrome.

The high burden of long-term symptoms up to one year after the infection in a wide range of patients, with a slight increase compared to that emerged at 6 months, confirm both the possible fluctuation of symptoms and the increased awareness of patients regarding the post-COVID-19 syndrome (15, 16). Available studies documented different rates of persistent symptoms after the onset of COVID-19 in different settings (2, 17). Numerous multisystem symptoms were reported, although rheumatological, anosmia/dysgeusia, fatigue, dyspnoea and psychiatric disorders were the most common (2, 18). Interestingly, at 12 months we found a significant increase in rheumatological, psychiatric and ocular symptoms compared with those reported at 6 months; in contrast, there was a significant decrease in neurological symptoms (3, 18). This delayed increase in rheumatological symptoms may confirm the role of persistent immune-mediated mechanisms, confirming the potential of SARS-CoV-2 to trigger autoimmune manifestations (2, 4). Mental health issues have been reported more frequently than in those recovering from other infectious diseases, possibly due to the traumatic effects of the COVID-19 pandemic on mental health (16, 19) .

The potential immune- mediated hypotheses of long COVID-19 remains uncertain (7, 20). Vaccination against SARS-CoV-2 is a leading strategy to change the course of the COVID-19 pandemic worldwide, reducing the risk of infection, that of severe complications and the long-term effects in the case of a breakthrough infection (8, 9, 21). Moreover, vaccines have shown to increase immunogenicity, antibody titres, and reactogenicity in individuals with past infection compared with naive patients (22). Vaccine hesitancy of previously infected patients and long-haulers might be due to the belief that, having developed a dysregulated response to natural infection, that may be exacerbated by vaccination, and also to the perception that protection is acquired with previous infection, as observed in our cohort (11, 23).

However, a limited evidence with conflicting results is available on the potential impact of vaccination on post-COVID-19 symptoms (7, 10, 11). Our findings suggest vaccination is not associated with worsened symptoms as when comparing vaccinated and unvaccinated patients, symptoms were mostly improved or unchanged. Of interest, we only found an improvement in hair loss and worsening of ocular symptoms among unvaccinated patients. Telogen effluvium

is a disorder induced by a wide variety of endogenous and exogenous factors, including COVID-19 infection (2). Ocular morbidities in long-haulers are an emerging problem that needs to be further studied (24).

Knowledge concerning humoral immune response to SARS-CoV-2 and its relationship with post-COVID-19 syndrome is still incomplete (5, 6, 25-27). We found a significant association between growing titres of non-RBD SARS-CoV-2 antibodies after natural infection and post-COVID-19 syndrome in the prolonged follow-up. In contrast, the presence and persistence of RBD IgG stimulated by the vaccine in patients with hybrid immunity were not associated with post-COVID-19 compared with patients with natural infection. The immune response induced by vaccines is a highly targeted response to the Spike protein of the SARS-CoV-2 virus and may help the immune system to tackle the possible viral reservoir, reducing the chance of non-specific immune reactions and resetting the immune response (22, 26, 28, 29). In contrast, the response triggered by natural infection is broader and may stimulate an excessive or dysregulated allo- and autoimmune response and uncontrolled inflammatory activity (22, 30). Based on our data on vaccination and humoral response, the SARS-CoV-2 vaccination should be recommended in patients with a history of previous COVID-19 because further vaccine immune stimulation may not exacerbate sequalae nor produce an altered humoral response. Moreover, long-COVID-19 patients would benefit from vaccination to reduce their risk of further infection and to avoid the risk of a vicious immune circle (31, 32).

This study has several limitations. It has involved a single-centre study including patients cared for in the first wave, limiting its generalizability given that the emergence of SARS-CoV-2 variants of concern may affect the clinical presentations, the serological responses, the occurrence and the severity of long-COVID. Moreover, a 20% drop-off rate between the 6 and 12 month interviews was observed and only COVID-19 patients were included, thus lacking in a control group (16). Furthermore, given that vaccine programme in Italy prioritized HCWs and elderly patients this might have introduced a gender bias (23). Symptoms were self-reported and subjectivity may have affected the findings. Additionally, the test accuracy, positivity cut-offs and kinetics of antibodies may be assay-dependent, and measuring antibodies may be a limited indicator of immunity without the determination of cellular immunity.

In conclusion, patients with different degrees of COVID-19 severity, cared for in the first wave of the pandemic, perceive a high burden of post-COVID-19 sequelae with multi-organ clinical manifestations up to one year after the onset. Vaccination does not seem to stimulate the appearance of symptoms, suggesting that individuals with a history of acute COVID-19 would benefit from SARS-CoV-2 vaccination. Persistently high non-RBD SARS-CoV-2 IgG titres induced by natural infection are associated with post-COVID-19 syndrome, whereas the presence and the persistence of RBD SARS-CoV-2 IgG antibodies stimulated by the vaccine in patients with hybrid immunity are not associated with post-

COVID-19 compared to those unvaccinated. A better understanding of the potential role of vaccination and humoral immune responses to SARS-CoV-2 is needed to inform the development of preventive and treatment strategies in the chronic phase of COVID-19.

Funding

This research was funded by PRIN 2017 n.20178S4EK9 – "Innovative statistical methods in biomedical research on biomarkers: from their identification to their use in clinical practice".

Conflicts of interest

Maddalena Peghin reports receiving grants and personal fees from Pfizer, MSD, Menarini and Dia Sorin outside the submitted work. Carlo Tascini has received grants in the last two years from Correvio, Biotest, Biomerieux, Gilead, Angelini, MSD, Pfizer, Thermofisher, Zambon, Shionogi, Avir Pharma and Hikma outside the submitted work.
The other authors have no conflict of interest to declare.

Authors'contributions

Conceptualization: M.Peghin, A.Palese, M.Isola, C. Tascini; Methodology: M.Peghin, A.Palese, M.De Martino, M.Isola, C. Tascini; Software: M.De Martino, M.Isola; Validation: M.De Martino, M.Isola; Formal Analysis: M.De Martino M.Isola; Investigation: M.Peghin, A.Palese, M.Isola, M.De Martino, C. Tascini; Resources: M.Fabris, F.Curcio, A.Sartor; Data curation: V.Gerussi, E.Graziano, G.Bontempo, D. D' Elia, F. Dellai F.Marrella ; Writing – Original Draft : M.Peghin, A.Palese, M.De Martino, M.Isola; Writing – Review & Editing : M.Peghin , A.Palese, M.Isola, M.De Martino, M.Fabris, F.Curcio, C. Tascini; Visualization: M.Peghin, M.Isola, M.De Martino; Supervision: M .Peghin , A.Palese , M.Isola, A.Sartor C. Tascini; Project Administration: M.Peghin, A.Palese , M.Isola, F.Curcio, C. Tascini ; Funding acquisition : M.Isola

Acknowledgements

The authors would like to thank all clinical and nursing staff who cared for the patients at Udine Infectious Disease Clinic during hospitalization and ambulatory management. The authors are grateful to all patients for their collaboration.

References

1. Adeloye D, Elneima O, Daines L, Poinasamy K, Quint JK, Walker S, et al. The long-term sequelae of COVID-19: an international consensus on research priorities for patients with pre-existing and new-onset airways disease. Lancet Respir Med. 2021;9(12):1467-78.
2. Nasserie T, Hittle M, Goodman SN. Assessment of the Frequency and Variety of Persistent Symptoms Among Patients With COVID-19: A Systematic Review. JAMA Netw Open. 2021;4(5):e2111417.
3. Wu X, Liu X, Zhou Y, Yu H, Li R, Zhan Q, et al. 3-month, 6-month, 9-month, and 12-month respiratory outcomes in patients following COVID-19-related hospitalisation: a prospective study. Lancet Respir Med. 2021;9(7):747-54.

4. Ortona E, Malorni W. Long COVID: to investigate immunological mechanisms and sex/gender related aspects as fundamental steps for tailored therapy. Eur Respir J. 2022;59(2).
5. Peghin M, Palese A, Venturini M, De Martino M, Gerussi V, Graziano E, et al. Post-COVID-19 symptoms 6 months after acute infection among hospitalized and non-hospitalized patients. Clin Microbiol Infect. 2021;27(10):1507-13.
6. Pereira C, Harris BHL, Di Giovannantonio M, Rosadas C, Short CE, Quinlan R, et al. The Association Between Antibody Response to Severe Acute Respiratory Syndrome Coronavirus 2 Infection and Post-COVID-19 Syndrome in Healthcare Workers. J Infect Dis. 2021;223(10):1671-6.
7. Arnold DT MA, Samms E, Stadon L, Maskell NA, Hamilton FW. Are vaccines safe in patients with Long COVID? A prospective observational study. MedRxiv. 2021.
8. Massey D BD, Krumholz H. Breakthrough Symptomatic COVID-19 Infections Leading to Long Covid: Report from Long Covid Facebook Group Poll. MedRxiv. 2021.
9. Antonelli M, Penfold RS, Merino J, Sudre CH, Molteni E, Berry S, et al. Risk factors and disease profile of post-vaccination SARS-CoV-2 infection in UK users of the COVID Symptom Study app: a prospective, community-based, nested, case-control study. Lancet Infect Dis. 2022;22(1):43-55.
10. Ledford H. Do vaccines protect against long COVID? What the data say 2021 [Available from: https://www.nature.com/articles/d41586-021-03495-2.
11. Strain WD SO, Banerjee A, van der Togt V, Hishmeh L, Rossman J. The Impact of COVID Vaccination on Symptoms of Long COVID. An International Survey of People with Lived Experience of Long COVID. 2021.
12. Powers JH, 3rd, Howard K, Saretsky T, Clifford S, Hoffmann S, Llorens L, et al. Patient-Reported Outcome Assessments as Endpoints in Studies in Infectious Diseases. Clin Infect Dis. 2016;63 Suppl 2:S52-6.
13. NICE. COVID-19 rapid guideline: managing the long- term effects of COVID-19 2022 [Available from: https://www.nice.org.uk/guidance/ng188/resources/covid19-rapid-guideline-managing-the-longterm-effects-of-covid19-pdf-51035515742.
14. Peghin M, De Martino M, Fabris M, Palese A, Visintini E, Graziano E, et al. The Fall in Antibody Response to SARS-CoV-2: a Longitudinal Study of Asymptomatic to Critically Ill Patients Up to 10 Months after Recovery. J Clin Microbiol. 2021;59(11):e0113821.
15. Tool to Assess Risk of Bias in Cohort Studies : Cochrane Review; [Available from: https://methods.cochrane.org/sites/methods.cochrane.org.bias/files/public/uploads/Tool%20to%20Assess%20Risk%20of%20Bias%20in%20Cohort%20Studies.pdf
16. Matta J, Wiernik E, Robineau O, Carrat F, Touvier M, Severi G, et al. Association of Self-reported COVID-19 Infection and SARS-CoV-2 Serology Test Results With Persistent Physical Symptoms Among French Adults During the COVID-19 Pandemic. JAMA Intern Med. 2022;182(1):19-25.
17. Liu T, Wu D, Yan W, Wang X, Zhang X, Ma K, et al. Twelve-month systemic consequences of COVID-19 in patients discharged from hospital: a prospective cohort study in Wuhan, China. Clin Infect Dis. 2021.
18. Joshee S, Vatti N, Chang C. Long-Term Effects of COVID-19. Mayo Clin Proc. 2022;97(3):579-99.
19. Martillo MA, Dangayach NS, Tabacof L, Spielman LA, Dams-O'Connor K, Chan CC, et al. Postintensive Care Syndrome in Survivors of Critical Illness Related to Coronavirus Disease 2019: Cohort Study From a New York City Critical Care Recovery Clinic. Crit Care Med. 2021;49(9):1427-38.
20. Bergwerk M, Gonen T, Lustig Y, Amit S, Lipsitch M, Cohen C, et al. Covid-19 Breakthrough Infections in Vaccinated Health Care Workers. N Engl J Med. 2021;385(16):1474-84.
21. Taquet M DQ, Harrison PJ. Six-month sequelae of post-vaccination SARS-CoV-2 infection: a retrospective cohort study of 10,024 breakthrough infections. MedRxiv. 2021.
22. Mangge H, Kneihsl M, Schnedl W, Sendlhofer G, Curcio F, Domenis R. Immune Responses against SARS-CoV-2-Questions and Experiences. Biomedicines. 2021;9(10).
23. Gerussi V, Peghin M, Palese A, Bressan V, Visintini E, Bontempo G, et al. Vaccine Hesitancy among Italian Patients Recovered from COVID-19 Infection towards Influenza and Sars-Cov-2 Vaccination. Vaccines (Basel). 2021;9(2).
24. Costa IF, Bonifacio LP, Bellissimo-Rodrigues F, Rocha EM, Jorge R, Bollela VR, et al. Ocular findings among patients surviving COVID-19. Sci Rep. 2021;11(1):11085.

25. Durstenfeld MS PM, Kelly JD, Win S, Swaminathan S, Li D, et al. Role of antibodies, inflammatory markers, and echocardiographic findings in post-acute cardiopulmonary symptoms after SARS-CoV-2 infection. MedRxiv. 2021.
26. Massey D BD, Akrami A, Assaf G, Davis H, Harris K, McCorkell L, Ring AM, Schulz WL, Wei H, Krumholz HM, Iwasaki A. Change in Symptoms and Immune Response in People with Post-Acute Sequelae of SARS-Cov-2 Infection (PASC) After SARS-Cov-2 Vaccination. MedRxiv. 2021.
27. Garcia-Abellan J, Padilla S, Fernandez-Gonzalez M, Garcia JA, Agullo V, Andreo M, et al. Antibody Response to SARS-CoV-2 is Associated with Long-term Clinical Outcome in Patients with COVID-19: a Longitudinal Study. J Clin Immunol. 2021;41(7):1490-501.
28. Zhang J, Lin H, Ye B, Zhao M, Zhan J, Dong S, et al. One-year sustained cellular and humoral immunities of COVID-19 convalescents. Clin Infect Dis. 2021.
29. Mishra PK BN, Ukey R, Datta P, Onyuka A, Handler D et al. Vaccination boosts protective responses and counters SARS-CoV-2-induced pathogenic memory B cells. MedRxiv. 2021.
30. Rosadas C, Randell P, Khan M, McClure MO, Tedder RS. Testing for responses to the wrong SARS-CoV-2 antigen? Lancet. 2020;396(10252):e23.
31. Cavanaugh AM, Spicer KB, Thoroughman D, Glick C, Winter K. Reduced Risk of Reinfection with SARS-CoV-2 After COVID-19 Vaccination - Kentucky, May-June 2021. MMWR Morb Mortal Wkly Rep. 2021;70(32):1081-3.
32. Bozio CH, Grannis SJ, Naleway AL, Ong TC, Butterfield KA, DeSilva MB, et al. Laboratory-Confirmed COVID-19 Among Adults Hospitalized with COVID-19-Like Illness with Infection-Induced or mRNA Vaccine-Induced SARS-CoV-2 Immunity - Nine States, January-September 2021. MMWR Morb Mortal Wkly Rep. 2021;70(44):1539-44.

Table 1. Baseline characteristics at the COVID-19 onset at the overall level and according to the vaccination status after 12 months

	Overall (n=479)	Vaccinated (n = 132)	Unvaccinated (n = 347)	p-value
Gender, n (%)				< 0.001
Female	252 (52.6)	94 (71.2)	158 (45.5)	
Male	227 (47.4)	38 (28.8)	189 (54.5)	
Age group, n (%) years				0.061
18–40	107 (22.3)	33 (25.0)	74 (21.3)	
41–60	205 (42.8)	64 (48.5)	141 (40.6)	
> 60	167 (34.9)	35 (26.5)	132 (38.0)	
Ethnicity, n/N (%)				0.360
Native Italian	422/457 (92.3)	112/125 (89.6)	310/332 (93.4)	
European	327457 (7.0)	12/125 (9.6)	20/332 (6.0)	
Non-European	3/457 (0.7)	1/125 (0.8)	2/332 (0.6)	
Smoking habit, n/N (%)				0.295
Non-smoker	310/477 (65.0)	81/131 (61.8)	229/346 (66.2)	
Smoker	68/477 (14.3)	24/131 (18.3)	44/346 (12.7)	
Ex-smoker	99/477 (20.7)	26/131 (19.9)	73/346 (21.1)	
Alcohol habit, n/N (%)				0.430
Non-drinker	238/476 (50.0)	70/130 (53.8)	168/346 (48.5)	
Drinker	235/476 (49.4)	60/130 (46.2)	175/346 (50.6)	
Alcohol use disorder	3/476 (0.6)	0/130 (0.0)	3/346 (0.9)	
Work, n/N (%)				< 0.001
HCWs	102/443 (23.0)	73/120 (60.9)	29/323 (9.0)	
Work in contact with public	84/443 (19.0)	13/120 (10.8)	71/323 (22.0)	
Work not in contact with the public	121/443 (27.3)	14/120 (11.7)	107/121 (33.1)	
Retired	81/443 (18.3)	10/120 (8.3)	71/121 (22.0)	
Other	55/443 (12.4)	10/120 (8.3)	45/121 (13.9)	
Co-morbidities, number, n (%)				0.160
0	230 (48.0)	64 (48.5)	166 (47.8)	
1	135 (28.2)	35 (26.5)	100 (28.8)	
2	66 (13.8)	25 (18.9)	41 (11.8)	
3	31 (6.5)	5 (3.8)	26 (7.5)	
≥ 4	17 (3.5)	3 (2.3)	14 (4.0)	
Co-morbidities, n/N (%)				
Hypertension	106/468 (22.6)	25/128 (19.5)	81/340 (23.8)	0.323
Obesity	78 (16.3)	22/132 (16.7)	56/347 (16.1)	0.889
Diabetes	25/475 (5.3)	6/130 (4.6)	19/345 (5.5)	0.698
Chronic respiratory disease^	17/475 (3.6)	6/130 (4.6)	11/345 (3.2)	0.421
Cardiovascular disease*	7/475 (1.5)	2/130 (1.5)	5/345 (1.4)	1.000
Liver disease	9/475 (1.9)	2/130 (1.5)	7/345 (2.0)	1.000
Psychiatric disorders°	5 (1.0)	1 (0.8)	4 (1.1)	1.000
Renal impairment	0/475 (0.0)	0/132 (0.0)	0/345 (0.0)	
Under chronic medication, n/N (%)				0.555
Yes	227/473 (48.0)	60/131 (45.8)	167/342 (48.8)	
No	246/473 (52.0)	71/131 (54.2)	175/342 (51.2)	

COVID-19, Coronavirus Disease 2019; HCWs, health-care workers; n, number, N, number as a denominator.
^Pulmonary disease: asthma, chronic obstructive pulmonary disease.
*Cardiovascular disease: heart failure, ischaemic heart disease, tachyarrhythmias, valvular heart disease, venous thromboembolism.
° Depression, anxiety.

Table 2. Clinical presentation at acute at the COVID-19 onset at the overall level and according to the vaccination status after 12 months

	Overall (n = 479)	**Vaccinated** (n = 132)	**Unvaccinated** (n = 347)	**p-value**
Acute COVID-19 severity*, n/N (%)				0.005
Asymptomatic	38/477 (8.0)	19/132 (14.4)	19/345 (5.5)	
Mild	323/477 (67.7)	86/132 (65.1)	237/345 (68.7)	
Moderate, severe and critical	116/477 (24.3)	27/132 (20.5)	89/345 (25.8)	
Symptoms at onset, number, n (%)				0.229
0	66 (13.8)	26 (19.7)	40 (11.5)	
1	66 (13.8)	15 (11.4)	51 (14.7)	
2	97 (20.2)	25 (18.9)	72 (20.7)	
3	74 (15.4)	20 (15.2)	54 (15.6)	
4	76 (15.9)	23 (17.4)	53 (15.3)	
≥ 5	100 (20.9)	23 (17.4)	77 (22.2)	
Management, n (%)				0.281
Outpatients	340 (71.0)	99 (75.0)	241 (69.4)	
Inpatients				
Ward^	118 (24.6)	30 (22.7)	88 (25.4)	
ICU	21 (4.4)	3 (2.3)	18 (5.2)	
Length of in-hospital stay, days, median (IQR)	7 (3-11)	6.5 (2–11)	7 (4–12)	0.341
Viral shedding, days, median (IQR)	19 (14-25)	18.5 (14–26)	20 (14–25)	0.631
Ct-values, median (IQR)	28.8 (24-33)	28.9 (23.7–32)	28.7 (24–33.5)	0.611
Post-COVID-19 syndrome 6 months, n (%)	201 (42.0)	44 (33.3)	157 (45.2)	0.018
Number of Post-COVID-19 symptoms °median (IQR)	1 (1-2)	2 (1–2)	1 (1–2)	0.084

COVID-19, Coronavirus Disease 2019; ct, cycle threshold; IQR, interquartile range; ICU, intensive care unit; n, number, N, number as a denominator.

* asymptomatic; mild (without pneumonia); moderate (with pneumonia); severe (with severe pneumonia); critical including Acute Respiratory Distress Syndrome (ARDS), sepsis and/or septic shock [32].

^ Infectious Disease or Pneumology Department.

° at 6 months

Table 3. Post-COVID-19 symptoms at 12 months compared with post-COVID-19 symptoms at 6 months stratified according to the vaccination status

	Vaccinated (n = 132)	Unvaccinated (n = 347)	p-value
Vaccine, n/N (%)			
Pfizer	114/126 (90.5)		
Moderna	4/126 (3.2)		
Astrazeneca	7/126 (5.6)		
J&J	1/126 (0.8)		
Post-COVID syndrome§			0.209
Unaffected + Unchanged	87 (65.9)	247 (71.2)	
Worsened	30 (22.7)	55 (15.8)	
Improved	15 (11.4)	45 (13.0)	
Post-COVID symptoms, n (%)			0.604
0	73 (55.3)	180 (51.9)	
1	27 (20.4)	65 (18.7)	
2	17 (12.9)	42 (12.1)	
3	7 (5.3)	27 (7.8)	
4	1 (0.8)	11 (3.2)	
≥5	7 (5.3)	22 (6.3)	
Fatigue			0.616
Unaffected + Unchanged	116 (87.9)	294 (84.7)	
Worsened	5 (3.8)	20 (5.8)	
Improved	11 (8.3)	33 (9.5)	
Anosmia/dysgeusia			0.947
Unaffected + Unchanged	117 (88.6)	306 (88.2)	
Worsened	8 (6.1)	20 (5.8)	
Improved	7 (5.3)	21 (6.0)	
Dyspnea			0.965
Unaffected + Unchanged	118 (89.4)	311 (89.6)	
Worsened	8 (6.1)	22 (6.3)	
Improved	6 (4.5)	14 (4.1)	
Cough			0.507
Unaffected + Unchanged	127 (96.2)	333 (96.0)	
Worsened	4 (3.0)	7 (2.0)	
Improved	1 (0.8)	7 (2.0)	
Chest pain			0.544
Unaffected + Unchanged	127 (96.2)	338 (97.4)	
Worsened	4 (3.0)	8 (2.3)	
Improved	1 (0.8)	1 (0.3)	
Headache			0.175
Unaffected + Unchanged	120 (90.9)	330 (95.1)	
Worsened	7 (5.3)	12 (3.5)	
Improved	5 (3.8)	5 (1.4)	
Rheumatological disorders			0.104
Unaffected + Unchanged	121 (91.6)	298 (85.9)	
Worsened	10 (7.6)	34 (9.8)	
Improved	1 (0.8)	15 (4.3)	
Gastrointestinal disorders			0.340
Unaffected + Unchanged	124 (93.9)	334 (96.2)	
Worsened	5 (3.8)	10 (2.9)	
Improved	3 (2.3)	3 (0.9)	
Cutaneous lesions			0.627
Unaffected + Unchanged	129 (97.7)	331 (95.4)	
Worsened	1 (0.8)	4 (1.1)	
Improved	2 (1.5)	12 (3.5)	

Hair loss			0.033
Unaffected + Unchanged	130 (98.5)	324 (93.4)	
Worsened	2 (1.5)	10 (2.9)	
Improved	0 (0)	13 (3.7)	
URTI symptoms			0.614
Unaffected + Unchanged	129 (97.7)	341 (98.3)	
Worsened	1 (0.8)	4 (1.1)	
Improved	2 (1.5)	2 (0.6)	
Ocular symptoms			0.021
Unaffected + Unchanged	127 (96.2)	327 (94.2)	
Worsened	3 (2.3)	20 (5.8)	
Improved	2 (1.5)	0 (0)	
Neurological disorders			0.707
Unaffected + Unchanged	120 (90.9)	308 (88.8)	
Worsened	1 (0.8)	7 (2.0)	
Improved	11 (8.3)	32 (9.2)	
Psychiatric disorders			0.505
Unaffected + Unchanged	117 (88.6)	293 (84.4)	
Worsened	10 (7.6)	36 (10.4)	
Improved	5 (3.8)	18 (5.2)	

COVID-19, Coronavirus Disease 2019; J&J, Johnson and Johnson; URTI, upper respiratory tract infection.

Table 4. SARS-CoV-2 RBD IgG and non-RBD IgG antibodies after natural infection and vaccination in patients with or without post-COVID-19 syndrome

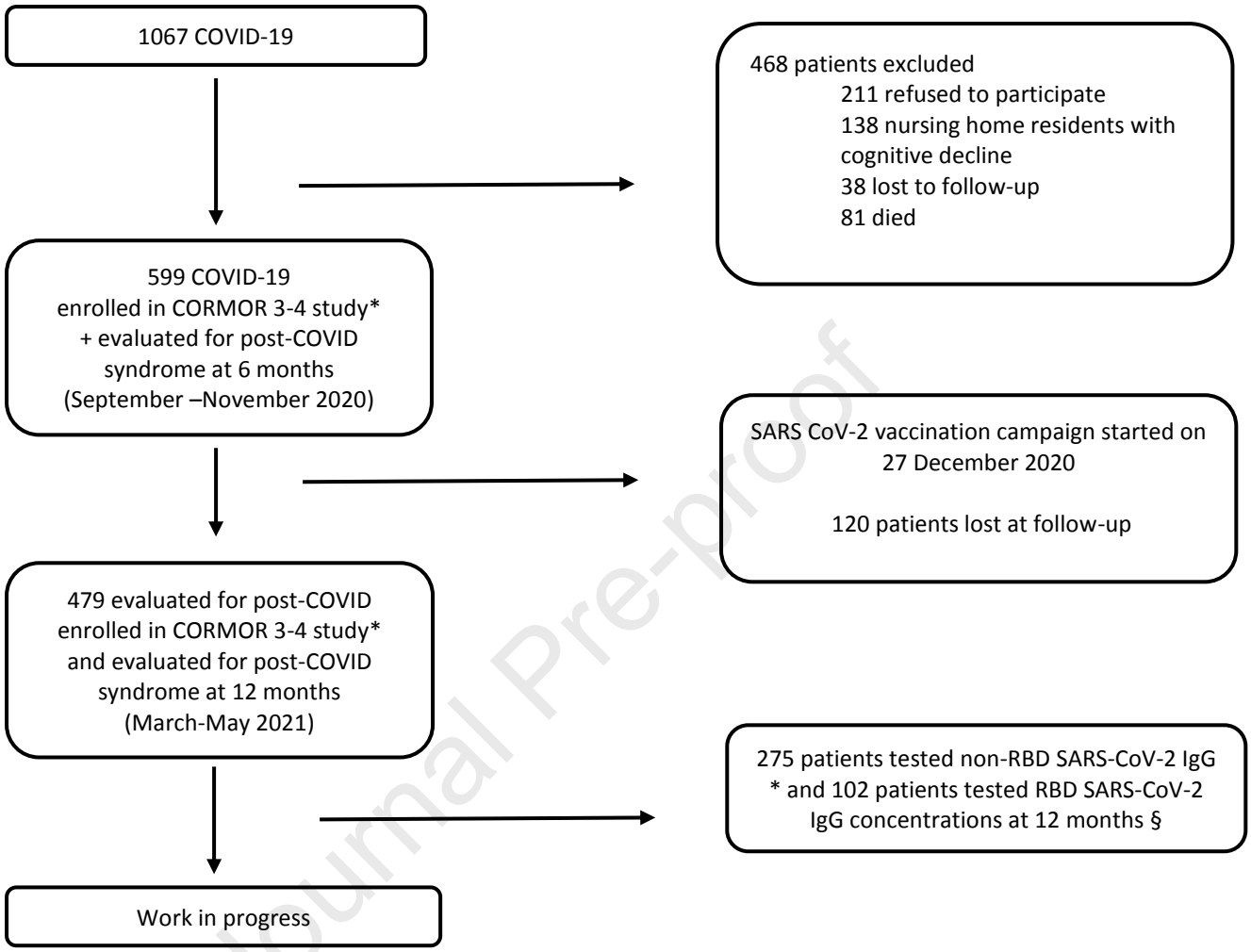
	Post-COVID-19 syndrome				p-value*
	Yes (N=153)		No (N=122)		
Non-RBD IgG at 12 months^, median (IQR)	22 (9.7–37.2)		14.1 (5.4–31.3)		**0.009**
	Vaccinated	**Unvaccinated**	**Vaccinated**	**Unvaccinated**	
RBD IgG at 12 months°, n/N(%)					0.451
< 0.9	0/31 (0.0)	2/23 (8.7)	0/27 (0.0)	0/21 (0.0)	
0.9–2500	3/31 (9.7)	19/23 (82.6)	3/27 (11.1)	21/21 (100)	
> 2500	28/31 (90.3)	2/23 (8.7)	24/27 (88.9)	0/21 (0)	

COVID-19, Coronavirus Disease 2019; n, number, N, number as a denominator, IQR, interquartile range; RBD, receptor binding domain; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.

^ Value available for 275 patients; °value available for 102 patients

*comparison between Post-COVID-19 Syndrome Yes/No

Figure 1. Flow diagram of in- and out- COVID-19 patients included in the post-COVID-19 syndrome study at 6-12 months and serological follow-up up to May 2021



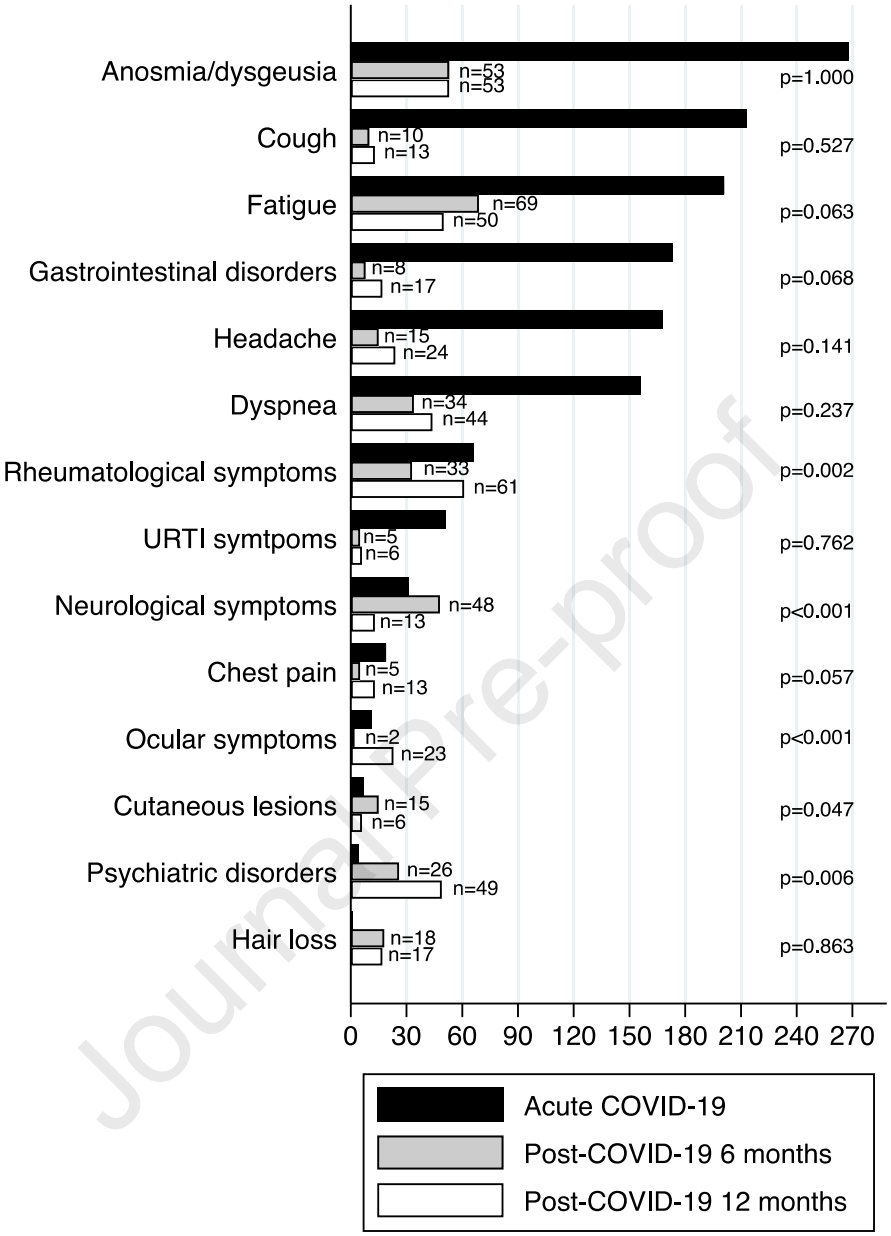
COVID-19, Coronavirus Disease 2019; RBD, receptor binding domain; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2

Legend: CORMOR 3-4 study

* non-RBD SARS-CoV-2 IgG antibodies (iFlash®) concentrations were measured at the serological follow-up visits each month (+/- 15 days) after symptom onset during the first four months, and every month up to 12 months (+/- 15 days), from March 2020 to May 2021. Among the 479 patients, only 275 were evaluated at 12 months.

§ RBD SARS-CoV-2 IgG antibodies (Roche®) at 12 months after the onset of symptom (+/- 60 days). Patients were categorized as vaccinated/hybrid immunity if they had received the vaccine at least two weeks before the interview.

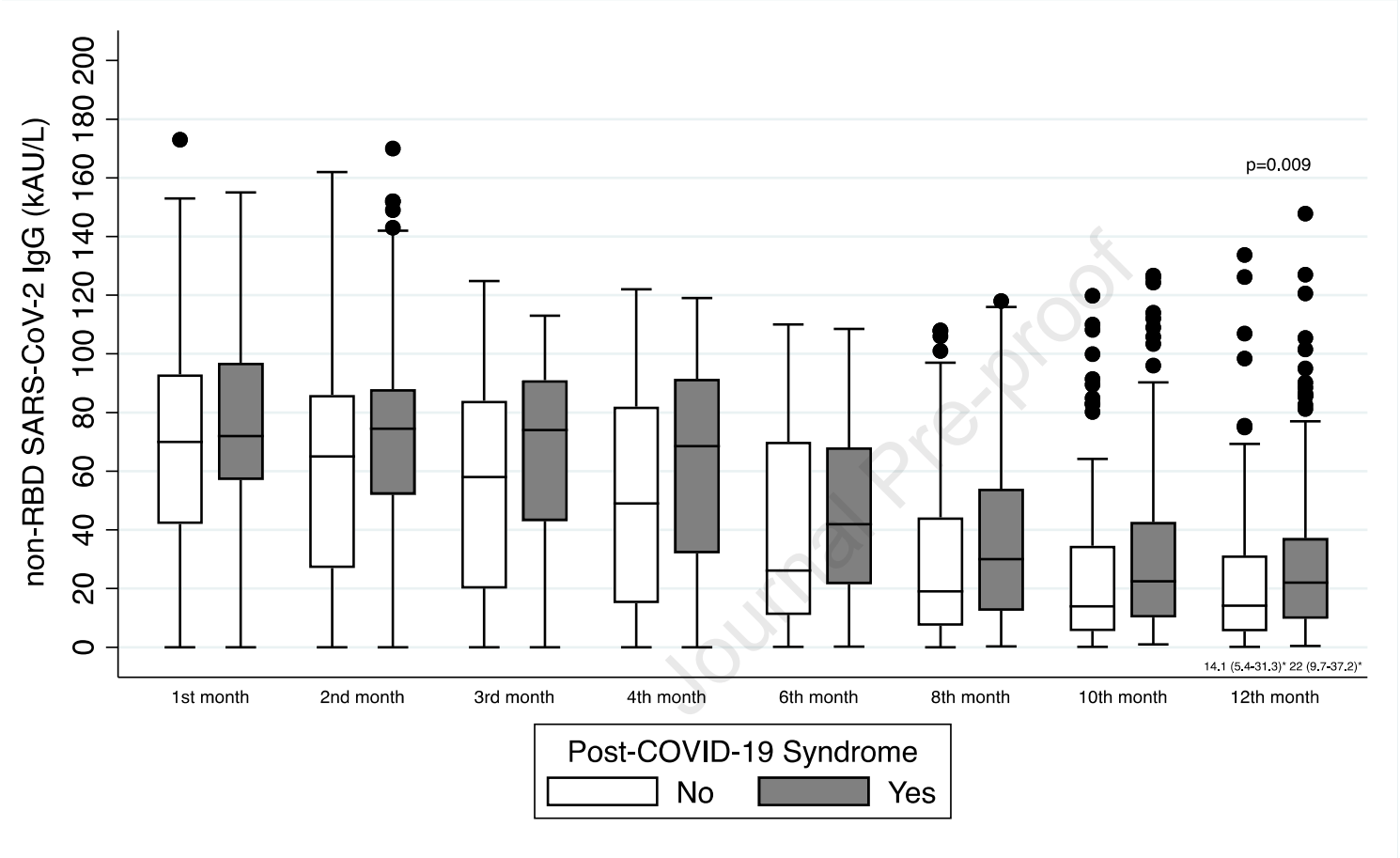
Figure 2. Acute- and post- COVID-19 related sympthoms at 6 and 12 months



*p refers to post-COVID-19 sympthoms at 6 and 12 months.

COVID-19, Coronavirus Disease 2019; URTI, upper respiratory tract infection.

Figure 3. Serological evolution againts SARS-CoV-2 measured with non-RBD SARS-CoV-2 IgG in patients with or without post-COVID-19 syndrome at 12 months.



COVID-19, Coronavirus Disease 2019; RBD, receptor binding domain; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2.